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EFFECT OF MONOMETHYLHYDRAZINE ON INSULIN LEVELS IN RATS

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

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FOR THE COMMANDER

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Monomethylhydrazine (MMH) causes a hyperglycemic response in rats although the exact mechanism is not known. There have been some data reported indicating that a decrease in insulin release following MMH exposure may be a causative factor. The studies described here were done to determine the effect of MMH on plasma glucose, plasma insulin, and liver glycogen levels in fasted and glucose stimulated rats. There was a hyperglycemic response but no change in insulin levels. MMH also appears to interfere with glycogenesis in rats given exogenous glucose. The effect of MMH on glucose metabolism is probably due to a block in

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anaerobic glycolysis.

PREFACE

This report represents research performed by the Toxicology Branch, Toxic Hazards Division, Aerospace Medical Research Laboratory from August 1974 to February 1976. The research was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202, "Procedures for Diagnosis and Treatment of Air Force Exposure Cases," Work Unit 63020215.

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INTRODUCTION

It has been recognized for many years that hydrazine and hydrazine derivatives affect carbohydrate metabolism (Underhill, 1914, 1915). Hydrazine causes hypoglycemia, an increase in free fatty acids, accumulation of pyruvate and lactate, and glycogen depletion (Fortney, 1966). Conversely, monomethylhydrazine (MMH) is reported to cause hyperglycemia (Dost et al., 1973). The mechanism for this effect is not clear although several theories have been advanced.

There has been some evidence reported suggesting a relationship between MMH-induced hyperglycemia and decreased insulin levels. Potter et al. (1969) found that hydrazine and monamine oxidase inhibitors (MAOI) containing hydrazine groups caused a decrease in both plasma glucose and insulin, whereas non-hydrazine MAOI's increased plasma glucose. In studies investigating the mechanism of hydrazine-induced hypoglycemia and hypoinsulinemia, Aleyassine and Lee (1971) observed that MAOI's, with or without hydrazine groups, inhibit the increase in serum insulin levels in response to glucose administration in vivo and also block the release of insulin from pancreatic tissue in vitro. They suggested that the inhibition of insulin release is due to the intracellular accumulation of biogenic amines as a result of MAO inhibition. Since MMH is a monamine oxidase inhibitor (Wykes, 1966), the inhibition of insulin release could be a causative factor in MMH-induced hyperglycemia. Further evidence was the fact that insulin therapy coupled with pyridoxine was effective against MMH-induced hyperglycemia (Dost et al., 1973). The following experiments were done to determine if the hyperglycemia following MMH exposure was in response to low insulin levels.

METHODS

Reagent grade MMH was obtained from Matheson Coleman Bell. Male rats, Sprague Dawley CF Strain, weighing between 350-450 g were used. Four groups of rats, 10 per group, were fasted 18 hours and allowed water ad lib before use. Groups 1 and 3 were injected intraperitoneally (I.P.) with saline and Groups 2 and 4 with 16 mg MMH per kilogram (kg) body weight. Preliminary work showed the dose of MMH was not convulsigenic. Thirty minutes after injection, Groups 3 and 4 were given 300 mg glucose by gastric intubation. All rats were killed by cervical dislocation two hours after glucose intubation. Heparinized blood samples were taken from the heart, centrifuged and plasma removed for glucose and insulin determinations. Glucose was measured by the hexokinase method (Slein, 1963). Insulin was determined using a radioimmunoassay kit from Schwarz Mann Company; this is a modification of the dextran-coated charcoal immunoassay method described by Herbert et al. (1965). The livers were removed from all rats immediately after sacrifice and frozen in liquid nitrogen until glycogen levels were determined. Liver samples were weighed, homogenized in cold 10% trichloracetic acid and processed for glycogen content according to the method of Pfleiderer (1963).

A second experiment was done to determine the glucose tolerance of rats injected I.P. with saline or with 16 mg MMH/kg body weight. Thirty minutes after injection, six rats were sacrificed for baseline values. The rest were given 300 mg glucose by gastric intubation. Six control rats given saline and six rats given MMH were sacrificed at 30 minute, 1, 2 and 3 hour intervals after glucose intubation. Plasma insulin and glucose levels and liver glycogen levels were determined by the methods referenced.

RESULTS

The results of the first experiment on the effect of MMH on plasma glucose and insulin and liver glycogen in fasted rats and hyperglycemic rats are shown in Table 1. The glucose levels of control rats given saline alone

TABLE 1

Effect of MMH and MMH Plus Glucose on Plasma Glucose and Insulin and Liver Glycogen†

Treatment	Glucose mg/dl	Insulin μU/ml	Glycogen mg/g
Group 1, Saline	102 ± 4.0	34 ± 10	0.94 ± 0.34
Group 2, MMH	153 ± 14.0*	37 ± 7	1.62 ± 0.50
Group 3, Saline + Glucose	106 ± 2.5	24 ± 4	3.42 ± 1.08**
Group 4, MMH + Glucose	160 ± 11*	24 ± 3	1.30 ± 0.42

† Mean ± S.E. of Mean
* P > .01
** P > .05

and saline plus glucose were normal two hours after the glucose load. The rats injected with MMH and MMH plus glucose had significantly higher plasma glucose levels than the controls given saline and saline plus glucose, respectively. It can be seen that injection of MMH results in a significant rise in glucose in fasted or glucose-fed rats. The liver glycogen levels in all groups were low after an 18 hour fast. The only significant difference was between rats injected with saline and those given saline and glucose, reflecting the glycogenesis from the glucose load. The glycogen levels of the rats given MMH plus glucose and MMH alone were not significantly different. It appears possible that MMH blocks the conversion of glucose to glycogen, since there was no comparable increase in glycogen in the rats given MMH and glucose as there was in those given saline and glucose. The insulin levels showed no differences although there was a fairly wide variation within each group.

In order to follow the glucose and insulin levels over a period of time, a second experiment was done in which rats were given a glucose load

following injection with MMH or saline. Plasma glucose and insulin were determined at 30 minutes, 1, 2 and 3 hours. The results are shown in Table 2. The rats injected with saline had a peak glucose level at 30

TABLE 2

Effect of MMH on Plasma Glucose and Insulin and Liver Glycogen Following a Glucose Load†

	Time	Saline + Glucose	MMH + Glucose
Plasma Glucose mg/dl	0	104 ± 4	100 ± 5
	30 min	145 ± 7	132 ± 6
	1 hour	125 ± 4	130 ± 5
	2 hour	121 ± 6	149 ± 5
	3 hour	106 ± 7	141 ± 9
Plasma Insulin µl/ml	0	13 ± 1	9 ± 1
	30 min	17 ± 1	20 ± 4
	1 hour	15 ± 1	13 ± 2
	2 hour	15 ± 1	13 ± 1
	3 hour	14 ± 1	16 ± 1
Liver Glycogen mg/g	0	0.62 ± 0.43	0.19 ± 0.04
	30 min	0.80 ± 0.37	1.30 ± 0.79
	1 hour	4.18 ± 1.42*	0.50 ± 0.16*
	2 hour	6.14 ± 0.93**	0.33 ± 0.09**
	3 hour	4.49 ± 1.22	2.57 ± 0.82

† Mean ± S.E. of Mean
* P > .05
** P > .01

minutes with a return to baseline at three hours. The rats injected with MMH maintained an elevated glucose through the entire three-hour period. Again there was no significant difference between insulin levels of the controls and MMH-injected rats. The liver glycogen levels measured one and two hours after glucose load were significantly lower in MMH-injected rats than in controls. As in the first experiment, it would appear MMH may block glycogenesis. At three hours, the glycogen level in the MMH rats began to rise.

DISCUSSION

The results of these experiments confirm other work (Dost et al., 1973) reporting that injection of subconvulsive doses of MMH causes a hyperglycemic response in rats fasted or given a glucose load. However, this does not appear to be a response to a decreased release of insulin, since there was no significant difference in insulin levels between controls and MMH exposed rats. The hyperglycemic response may well be caused by an interference in

the glycolytic pathway. Using radiorespirometric techniques, Dost et al. (1973) have reported that the catabolism of ^{14}C glucose labelled in the 2, 3, 4, or 6 carbon positions to $^{14}CO_2$ is depressed in rats exposed to MMH. Since the turnover of pyruvate-2-^{14}C, acetate-2-^{14}C and butyric acid-1-^{14}C is not impeded by MMH, it would indicate there is no interference in the citric acid cycle and the block would be prior to pyruvate formation. Since fructose-1-^{14}C catabolism to glyceraldehyde and dehydroxy acetone is not significantly affected by MMH either, they conclude that the interference by MMH occurs in the glucose to triose portion of the glycolytic cycle. The pentose shunt also is relatively unaffected since glucose 1-^{14}C catabolism proceeds at a normal rate, indicating the block in glycolysis is probably at the phosphofructokinase or phosphohexoseisomerase steps.

In both experiments, the fact that there was no increase in liver glycogen after a glucose load in MMH injected rats as there was in the control rats suggests there is a block in the conversion of glucose to glycogen. Plasma glucose remains elevated and liver glycogen levels remain low until three to four hours after MMH injection. MMH is excreted fairly rapidly with a large percentage of the injected dose found in the urine and respiratory gases within the first four hours (Dost et al., 1966; Pinkerton et al., 1967). Three hours following the glucose load the glycogen levels begin to rise; indicating that the level of MMH is probably below the concentration necessary for interference with glycogenesis.

The hyperglycemia caused by MMH appears to be caused by a block in the glycolytic pathway probably at the phosphofructokinase step and an interference in the conversion of glucose to glycogen. Interference in insulin release does not seem to be a causative factor.

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